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Cold Comfort Pharm

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Abstract

Cooling of the skin has long been thought to be beneficial in pain states but intense cold is clearly noxious. Does cooling lead to pain or gain? Rapid progress in this controversy has been made since the discovery of specific ion channels of the TRP family that are activated by cooling of sensory nerve cells to below body temperature. This review focuses on the role of one of these, TRPM8, which has been implicated in cool sensation and cold pain by recent knockout mouse studies, but remarkably also appears capable of eliciting a novel analgesic gating control over noxious inputs in chronic pain states. We discuss hypothetical mechanisms that could bring about this composite profile. It is clear that new and highly selective agents will need to be developed to further evaluate the potential therapeutic opportunities offered by low temperature-sensitive TRP channels.

Introduction

It seems to be commonly understood that cooling an area of injury can relieve pain, but what is the scientific basis? Sports physiotherapists, dentists and medical practitioners have used cool sprays, mint oil and menthol for quite some time. Even before this, as far back as classical Greece, in the foundations of modern civilisation, the ancient Greek physician and 'father of medicine' Hippocrates (*circa*. 460-370 B.C.) and the personal physician to Marcus Aurelius, Galen (129-200 A.D.) reported that cutaneous cooling was effective as an analgesic remedy. Traditional medicine from China and Europe makes use of the natural cooling agent menthol and mint oils as analgesic therapies. Modern medicine has continued to make use of peripheral cooling to produce analgesia[1]. Menthol has also been shown to alleviate thermally-elicited pain in an experimental setting[2] and to exert an analgesic action in the mouse hot-plate and acetic acid writhing tests[3]. Despite the apparent success of menthol and cool in acute pain and inflammation, its effectiveness in chronic pain states has not yet been substantially established.

Humans have a highly developed temperature detection system which can perceive temperature changes as small as 1 deg C. Classic work by Hensel and Zotterman[4] indicated a role of specific receptors in mediating the transduction of cold sensation. Recent identification of the TRP (Transient Receptor Potential) family of ion channels in the nervous system, several of which respond to changes in temperature, has greatly advanced our understanding of this process.

Somatosensory systems convert environmental sensations via cutaneous afferent neurons that have free nerve endings in the skin capable of detecting physico-chemical stimuli and relay that information to the central nervous system. Indeed, it seems that distinct sets of such primary afferent neurons (with cell bodies in dorsal root ganglia, DRG) are specialized to respond to specific temperatures in the cold/cool to warm/hot range and transmit this information to the central nervous system.

Physiological pain detection (nociception) occurs via specialised cutaneous sensory neurons (nociceptors) which are afferents that are activated by noxious (painful) stimuli. They are functionally divided into two groups consisting of Aδ mechano-heat nociceptors and C-fibre polymodal nociceptors. Electrical stimulation of Aδ fibres evokes a rapid, sharp pain sensation (corresponding to 'first pain'), while stimulation of C-fibres produces the dull, diffuse or burning pain (corresponding to 'second pain'). Aδ and C fibre afferents also contain subpopulations that respond to cooling to innocuous cool temperatures of 15-30^{o}C or to noxious cold at 15^{o}C and below[5, 6]. Normal skin temperature is typically around 32^{o}C and activation of DRG fibers at temperatures of between 32^{o}C and 43^{o}C is perceived as warmth. Temperatures greater than 43^{o}C are perceived as noxious (painful) heat.

Chronic neuropathic pain can result from nerve damage of various origins, for example, by direct constriction, viral infection or due to diabetic or chemotherapeutic neurotoxicity. Ongoing chronic pain provides no benefit in terms of redirecting behaviour and severely reduces patients' quality of life. Laboratory models of peripheral nerve injury allow us to examine the underlying mechanisms that cause hypersensitive responses with the aim of identifying novel analgesic targets. Typical models can involve constriction of the sciatic nerve (chronic constriction injury, CCI or spinal nerve ligation, SNL), which result in behavioural hyperalgesia (heightened response to a painful stimulus) and allodynia (pain in response to innocuous stimuli). Models of demyelinating diseases and chronic inflammation are also used to establish whether there are common (or distinct) underlying factors that produce pain hypersensitivity following the different types of injuries.

This review aims to outline and evaluate the evidence for roles of TRP channel subtypes in cold sensation, in cold pain and in analgesia for chronic pain states. The particular focus is on a receptor for mild cooling, TRPM8, which has been

implicated in each of these roles from recent mutant mouse and antisense studies.

TRP channels in temperature detection

Members of the Transient Receptor Potential (TRP) family, the TRPV (vanilloid), TRPM (melastatin) and TRPA (ankyrin) receptors comprise the temperature-gated family of ion channels. Identification and cloning of the classic TRPV1 (VR1) vanilloid receptor marked the beginning of the process to identify TRP ion channels that were gated by warm and hot temperature and substances that elicit thermal sensations. Prior to this, sensitivity to capsaicin (the agent that produces the tingling heat perception in response to chili peppers) was a known feature of nociceptive Aδ and C fibres that correlated with their responsiveness to moderately raised temperatures of about 43°C. Subsequently, TRPV1 was identified as the channel responsible, as its activation was demonstrated in response to both moderate heat (43°C) and capsaicin[7]. This was the first demonstration of how sensory neurons may detect temperature. TRPV1 is expressed in the majority of Aδ/C peptidergic afferents and non-peptidergic IB4-positive afferents in the rat[8]. TRPV1-knockout mice are unresponsive to capsaicin and have reduced inflammation-induced thermal hyperalgesia[9, 10]. Although acute thermosensation has been reported as unaffected[10], thermal responses of afferent fibres, DRG cells and dorsal horn neurons are clearly impaired[9]. However, TRPV1 mutant mice retain responsiveness to high threshold noxious heat and at least some of the alternative thermoreceptors likely to be responsible have since been identified.

The closely-related channel, TRPV2 (VRL1), is a capsaicin-insensitive channel activated by noxious temperatures (activated above 52-55°C) in vitro[11]. TRPV2 is expressed predominantly by Aδ fibres which could represent a discrete population of Aδ mechano-heat receptors. TRPV3 (activation range 31-39°C) and TRPV4 (activated above 25°C) are sensitive to increasing innocuous warm

temperatures[12, 13]. TRPV3 is expressed by keratinocytes in the epidermis and its role in thermosensation is demonstrated by the marked deficits in responses to innocuous and noxious heat seen in TRPV3-null mice[14]. TRPV4 is found in both afferents and skin and contributes to both innocuous warm sensation and inflammation-induced thermal hyperalgesia[15, 16]. Interestingly, TRPV1 has also been detected in keratinocytes and in bladder epithelial cells[17]. Thus, some of these channels are not exclusive to sensory neurons and may thus subserve other as yet unidentified roles possibly involving trans-cellular information transfer to neurons.

Cold-responsive primary afferent fibres can be activated either by low threshold "cool" temperatures (approx 20-35°C)[18, 19] or by high-threshold noxious cold temperatures (<15°C), which are generally perceived as painful[5, 20]. A similar differentiation between the encoding of noxious and innocuous cold temperatures continues in the dorsal horn of the spinal cord. Neurons in the superficial dorsal horn that are specifically responsive to innocuous cool in primates receive input mainly from Aδ fibres[21, 22], whereas noxious cold-responsive cells are generally multireceptive, being also activated by heat and noxious pinch[23]. The ascending axons of cool-specific cells and the noxious cold-activated multireceptive cells have different conduction velocities and different thalamic terminations[24], as well as different morphologies. Subpopulations of thalamic neurons have also been identified that respond to cool, but not noxious cold, or alternatively to noxious cold[25]. Psychophysical studies further show differences between perception of cool and noxious cold, suggesting that the two signals are differentially processed before arrival at cortical levels[26].

TRPM8: properties of a molecular sensor of cooling

Major progress in understanding the basis for cool and cold temperature transduction was provided by the identification of TRPM8 (TRP melastatin family member 8, formerly known as CMR1), a Ca^{2+}-permeable ion channel that can be activated by cool temperatures (18-24°C)[27, 28]. It is also activated and sensitised

by menthol and other chemicals that elicit sensations of cool; e.g. eucalyptol. Notably, threshold temperatures reported for the activation of recombinant TRPM8 channels are consistently lower than those in native trigeminal menthol-sensitive neurons, suggesting that sensitivity is facilitated by endogenous factors in vivo[29, 30]. TRPM8 is selectively activated by the synthetic cooling agent, icilin, which is 200 times more potent than menthol[27, 28], although there is evidence that responses to icilin, but not menthol, may require concurrent elevation of cytosolic Ca^{2+} concentrations[31]. Icilin can also interact at higher concentrations with other channels such as TRPA1[32]. The WS series of compounds are derived from menthol and several such as WS-3 can evoke TRPM8-mediated Ca^{2+} entry[33]. WS-12 is reported as the highest-affinity TRPM8 ligand to date, but it can reduce the effects of menthol[34], so may act as a partial agonist. A number of other pharmacological agents have been described as activators of TRPM8 but their targeting specificity is not yet entirely clear[33-35]. In addition, it has been shown that several TRPV1 inhibitors such as BCTC and capsazepine inhibit TRPM8 too [30, 33, 36]. Some agents that activate other TRP channels such as 2-APB and URB597 inhibit TRPM8[37, 38]. Whereas some broad-spectrum inhibitors such as SKF96365 and Cu^{2+} - 1, 10-phenanthroline also inhibit TRPM8, others such as Ruthenium Red do not[30]. Ethanol is also an effective TRPM8 inhibitor[36, 39]. Natural herbal remedies such as peppermint oil or eucalyptus oil may contain as-yet-uncharacterised TRPM8 activators, but this remains to be tested. The field lacks truly selective agents, particularly inhibitors, for TRPM8, and research in the area will be made more difficult by recent evidence that vanilloid activators of TRPV1, notably including capsaicin and resiniferatoxin (and also agonists/antagonists for CB_1 receptors) are highly effective blockers of TRPM8 activation by icilin[40].

It is unclear whether there may be an endogenous ligand for TRPM8. Endogenous and natural exogenous ligands for the TRPV1 receptor have been identified, eg anandamide and resiniferatoxin (see van der Stelt and di Marzo for review[41]). Endogenous ligands for TRPM8 in mammals have yet to be identified,

although endogenous phospholipid metabolites such as lysophospholipids and phosphatidylinositol 4,5-bisphosphate have been shown to facilitate TRPM8 channel function[42-44].

TRPM8 in cold temperature sensing

TRPM8 is expressed by a subset (~10-15%) of small diameter primary afferents in DRG and trigeminal ganglia and can be activated by cooling or by menthol[27, 28, 45-47]. While low doses of menthol produce a sensation of cooling and analgesia in chronic pain models[46], much higher doses produce a noxious burning sensation[48, 49], although it is not clear that TRPM8 is being specifically targeted at such doses[50, 51]. TRPM8 protein is normally co-expressed with peripherin (a marker of unmyelinated afferents) in the DRG. TRPM8 expression increases ipsilateral to CCI nerve injury and is also newly expressed in small myelinated (NF-200-positive), presumed-Aδ fibre cells[46, 52]. However, no alterations in TRPM8 expression were reported in the SNL model of nerve injury, or in a model of inflammation[53, 54]. Studies of primary afferent: dorsal horn neuron synapses found that TRPM8 activation by menthol and/or cooling increased mEPSC frequency but not amplitude[55-57], suggesting a location at presynaptic terminals of the dorsal horn. Indeed, TRPM8 is not thought to be expressed in spinal cord neurons, and any expression in spinal somatosensory pathways seems to originate entirely in the periphery[28, 46].

Key insights into the physiological roles of TRPM8 in cool sensation and pain processing have come from a cluster of studies on TRPM8-null mice and other recent work. The evidence is now overwhelming for a major role of TRPM8 in cool sensation. Experiments with in vitro skin-nerve preparation [58] showed that significant subpopulations of C- and Aδ- fibres were activated by cooling from 32^{o}C to 2^{o}C in wild-type mice, but not in TRPM8-knockouts, corresponding to an earlier in vivo report of C- afferent axons activated by the selective TRPM8 activator, icilin[46]. Furthermore, sensory ganglion cells from knockout mice showed greatly reduced Ca^{2+}-elevation responses to cooling (range 20^{o}C - 10 ^{o}C)

as well as to menthol and icilin[58-60]. Similarly, the behavioural shakes/jumps elicited by intraperitoneal injection of a high dose of icilin, suggested to be due to the cooling sensation of icilin[61], were greatly reduced in TRPM8-null mice[59, 60]. In addition, temperature selection studies using either two-plate or multi-range choice chambers showed a preference of wild-type mice for floor temperatures of around 30°C rather than those in the range downwards to 15°C[58-60]. Such preferences were clearly reduced in TRPM8-null mice, indicating that the avoidance behaviour was TRPM8-mediated. In this temperature range the stimuli are unlikely to be overtly noxious however, so the behaviours probably reflect reactions to cool perception rather than cold pain[62]. Similarly, the licking/flinching responses to skin cooling by acetone were consistently reduced in TRPM8-null mice[58-60], but measuring the skin temperature revealed that only innocuous temperatures > 15°C were reached[59].

The question of whether TRPM8 plays a part in the noxious properties of intense cold stimuli is harder to answer. Paw withdrawal responses from cold surfaces around 0°C are generally considered to reflect noxious stimulus-evoked defensive behaviours. However, TRPM8-knockout mice showed prolonged cold plate paw flick latencies in only one of three reports[59]. Even the TRPM8 knockout mice still find the cold-plate an aversive stimulus and a reduced (but still significant) subgroup of afferents from their skin respond to cold, indicating that there are clearly other sensors for noxious cold[58-60]. Possible mediators include TRPA1, but also other candidates[63], whose case is supported by evidence of cool-sensitive, menthol-insensitive afferents that fail to respond to TRPA1 activators[64] (see below). So, the evidence for TRPM8 as a direct mediator of cold pain per se is not strong, which in fact matches our intuitive understanding that modest cooling and contact with menthol or icilin at moderate doses really do not represent noxious experiences. Nevertheless, in CCI or CFA models of neuropathic or inflammatory pain, acetone application caused greatly increased behavioural responses that may reflect nociception and these were notably reduced in TRPM8-null mice[59]. Central sensitisation in these chronic pain models

leads to greatly accentuated sensory responses, so this does not necessarily suggest that the TRPM8-mediated acetone stimulus is in itself overtly noxious, but rather that it may be interpreted as so in this context. Cool allodynia following CCI has also been described [46] at skin temperatures less than 16°C but the involvement of TRPM8 was not investigated. This might suggest that in chronic pain states, attempts to activate TRPM8 would lead to pain. However, the selective TRPM8 activators, menthol and icilin consistently do not elicit pain responses or hypersensitivity at moderate doses, even in established pain states[3, 46, 65]. Thus TRPM8 activation alone appears insufficient to elicit cold pain.

TRPM8 activation: pain or gain?

Rather than eliciting cold pain by itself, it seems likely that TRPM8 may play some auxiliary role in this process. This would be consistent with the pro-nociceptive effect of intraplantar icilin in the cold plate test in wild-type but not TRPM8-null mice[60] and the sensitisation of reflex pain behaviours following topical application of high concentrations of icilin[46]. One hypothetical explanation might be that explicit cold pain actually requires the activation of dual inputs, both the direct mediator of noxious cold at <10°C (possibly TRPA1, see below) and also TRPM8. Noxious cold perception may thus require the activation of two distinct neural pathways for appropriate interpretation at higher centres. This will inevitably occur upon cooling from ambient temperatures down to the noxious temperature range since the procedure will clearly have surpassed the threshold for TRPM8 activation. Such a dual input logic gate might contribute to explaining the rather inconsistent observations with TRPM8 and TRPA1 knockouts in terms of noxious cold withdrawal responses. Dual knockout mice would help to address this hypothesis. However, the hyperalgesic effects of TRPA1 activation appear to be attenuated rather than facilitated by TRPM8 activation[46] arguing against this model, at least in terms of TRPA1 as cold mediator. An alternative hypothesis would be that there are two physiologically distinct subpopulations of TRPM8-containing afferents innervating the skin, one reflecting innocuous cool sensation and a second in which TRPM8 is expressed in nociceptors whose activation may

contribute to cold pain (Figure 1). A lower level of TRPM8 expression in the second group might explain the observation that pro-nociceptive effects of icilin are seen only at very high concentrations[46, 60]. TRPM8 is largely expressed in small afferents that are TRPV1-negative but TrkA-positive[28, 32, 66]. However, other reports describe from 10% up to 29% co-expression with TRPV1[54, 67] and there are a number of reports of trigeminal and DRG cells responding to both menthol and capsaicin, implying TRPM8/TRPV1 co-expression[27, 47, 67, 68]. Since TRPV1 characterises heat/acid-sensitive nociceptors, activation of TRPM8 in the same cells would presumably also be perceived as noxious. This could contribute to cold pain. The extent of such co-expression may depend on the precise origin of the afferent cells investigated and the in vivo or in vitro conditions under which they are studied. Any co-expression of TRPM8 with TRPV1 could further account for the paradoxical sensations of burning hot pain when a cold stimulus is applied following experimental A-fibre block or following damage to A-fibres in demyelinating diseases[69]. Nevertheless, it seems clear that a substantial proportion of TRPM8-positive cells are not classical nociceptors and may be able to exert a quite different functional influence on pain processing[46, 62].

Indeed, matching ancient and anecdotal descriptions of cooling- and menthol-induced analgesia, there is now clear evidence that we can gain from TRPM8 activation in chronic sensitised pain states, where it elicits a novel analgesic influence. Activation of TRPM8 using peripherally (topically) or centrally (intrathecally) applied TRPM8 activators, menthol and icilin, can prevent the sensitisation of reflex pain behaviours and the increased responsiveness of single dorsal horn neurons that are induced in the CCI model of chronic neuropathic pain[46]. Similarly, analgesia is elicited by mild cooling of the skin (20^{o}C -16^{o}C range), which would be appropriate temperatures for TRPM8 activation[46]. The icilin-induced analgesia is clearly mediated by TRPM8, as it is prevented by specific antisense knockdown of TRPM8. Icilin is also capable of producing analgesia in alternative chronic pain models, for example in Complete Freund's Adjuvant-induced inflammatory hypersensitivity and following

lysolecithin-induced demyelination. No effects on contralateral reflex responses were observed or in normal animals without a sensitised pain state. This, coupled with the requirement for only low doses of icilin, suggests that the use of TRPM8 activators for analgesia in chronic pain states may be associated with a good index of therapeutic specificity. In addition, new evidence from the TRPM8-null mice further supports the concept of an analgesic role of the channel in pain states. Modest cooling to 17^{o}C reduced early phase formalin-induced paw licking behaviours in wild type but not TRPM8-null mice[60]. The fact that late phase formalin responses were reduced by mild cooling in both wild type and TRPM8-null mice suggests not only that there may be additional molecular mediators of modest cooling but also that they too may contribute to cooling-induced analgesia.

Connecting cold inputs and pain processing

If the analgesia produced by cold temperatures is mediated by activation of specific cold-responsive afferents, what are the possible mechanisms of this action? How can cold fibres affect central processing of pain? Since icilin can both activate a subpopulation of fine afferents and can elicit analgesia, there are likely to be key changes occurring in the central nervous system (CNS). The first step in central processing of both pain and cold afferents is in the dorsal horn of the spinal cord. Glutamate receptors are well-established mediators of central sensitisation; the enhanced spinal synaptic transmission that underlies a wide range of chronic pain states. Could inhibitory glutamate receptors be underlying the centrally mediated component of this analgesia? The glutamate receptors are classed as NMDA, non-NMDA and metabotropic receptors. Although NMDA and non-NMDA (AMPA, kainate) receptors are exclusively involved in the expression and enhancement of excitatory transmission, metabotropic receptors can either be excitatory (Group I, mGluR1, 5) or inhibitory (Group II/III). Thus, it is possible that cold fibres could inhibit pain messages in the spinal cord by means of inhibitory metabotropic glutamate receptors. Agonists for inhibitory subtypes of Group II/III mGluRs cause reversal of CCI-induced mechanical and thermal

behavioural sensitisation[46]. So these receptors could potentially mediate an endogenous analgesic pathway relying on glutamate release.

Notably, blocking the activation of these receptors with selective mGluR Group II or III antagonists prevents the ability of icilin to reverse behavioural sensitisation, suggesting that the central analgesic influence of TRPM8 activation in neuropathic pain is mediated by mGlu Group II/III receptors. Also, when the Group II/III antagonist UBP 1112 was applied to dorsal horn neurons, it could prevent the reduction in noxious stimulus-induced firing that was caused by peripheral application of icilin[46]. Interestingly, the opioid receptor antagonist, naloxone had no effect on icilin analgesia, suggesting that this phenomenon is independent of the classical opioid analgesic system.

The existence of a modulatory pain system was proposed by Melzack and Wall[70] in the 'Gate Control' theory of pain, which proposed that spinal nociceptive transmission could be inhibited by non-nociceptive inputs. In the original 'Gate Control' theory, this inhibition was proposed to be produced by low-threshold mechanosensitive Aβ fibres, gating the input from nociceptive C and Aδ fibres. However, it now seems possible that innocuous cold-sensitive small-diameter afferents could gate the information from nociceptive afferents. There is evidence for presynaptic inhibition of nociceptive afferents produced by activity in other small-diameter afferents: in one recent study repetitive activation of sciatic Aδ fibres produced a presynaptically-mediated inhibition of saphenous C afferents[71], and it has been shown that Aδ fibre stimulation can cause a long-term depression of C fibre-evoked spinal field potentials[72]. Furthermore, a gating effect of innocuous cool-sensing afferents could be consistent with earlier observations, in that blockade of myelinated fibre input by selective conduction inhibition lowers the threshold for cold-induced pain, and results in the perception of cold pain as burning heat[73, 74]. Therefore it is possible that innocuous cold-sensitive fibres, which in humans are myelinated Aδ afferents, suppress the incoming information from cold-sensitive polymodal nociceptive C fibres and that

removal of this inhibition by selective conduction block or demyelination unmasks cold-induced burning pain. The observation that the hyperalgesia induced by TRPA1 activation can be attenuated by simultaneous activation of TRPM8[46] is consistent with this scheme.

The precise cellular arrangements that might underlie the hypothetical TRPM8-driven gating system in dorsal horn are of course unclear. TRPM8 activators can act presynaptically to facilitate excitatory transmitter release[55-57], although evidence for TRPM8-independent Ca^{2+} mobilisation by menthol[51] may complicate interpretation. TRPM8 activation can also lead, presumably by indirect means, to increased postsynaptic excitability of dorsal horn neurons[46]. The inhibitory mGlu Group II/III receptors that appear to mediate icilin analgesia within the dorsal horn can be localised both presynaptically and postsynaptically[75-77]. In addition, it appears that TRPM8-positive afferents, which are also characterised by cadherin-8 expression, form complex glomerular synapses, in which the core axonal bouton is surrounded by several dendritic and axonal processes[56]. Furthermore, the complexity of the different types of synaptic arrangement in superficial dorsal horn is exemplified in recent work by Lu and Perl[78]. This illustrates multiple inhibitory and excitatory influences of monoamines observed in different subpopulations of superficial dorsal horn neurons and emphasises the principle that TRPM8-induced analgesia may in fact derive from the integration of a number of diverse polysynaptic processes occurring within this region.

TRPA1 and other possible mediators of cold sensitisation

While TRPM8 and TRPV1 might be expressed in distinct primary afferent populations, it appears that another putative cold channel, TRPA1 (formerly known as ANKTM1) that is expressed in about 20% of DRG neurons[79] shows a 97% overlap with expression of TRPV1 but not TRPM8[66]. Thus it is tempting to suggest that there could be a population of cells that respond to both noxious heat and noxious cold stimuli, but do not detect innocuous cooling. TRPA1 has

been proposed to mediate detection of noxious cold, as TRPA1 is reported to be activated at temperatures below around 17°C, a temperature approaching pain for humans[32, 80]. However, there is conflicting evidence as to whether TRPA1 is the key mediator of noxious cold responses[81]. While TRPA1 knockdown reduced sensitised noxious cold responses following nerve injury[53], homozygous TRPA1 knockout mice surprisingly showed only partial (or no) attenuation of noxious cold withdrawal responses[82, 83]. TRPA1 was also proposed as a mediator of mechanotransduction in auditory stereocilia but knockout studies have failed to support this proposed role[82, 83]. TRPA1 is a menthol-insensitive channel that is activated by strong cold and noxious chemicals such as cinnamaldehyde and bradykinin[32, 84, 85]. TRPA1 is also a receptor for pungent isothiocyanates (which are found in wasabi and mustard) and for other natural products found in cinnamon, wintergreen, clove oil and garlic, such that TRPA1 may be involved in the inflammatory and vasodilator effects of these compounds[84, 85]. Recent evidence also indicates that TRPA1 may mediate the nociceptive actions of the industrial pollutant acrolein, and indeed underlie the formalin inflammatory pain model[82, 86]. Although there are some reports that TRPA1 does not respond to low temperatures[85, 87], the range of thermal and pungent stimuli that have been described for this channel strongly associate its activation with nociception. Activators of TRPA1 such as cinnamaldehyde and allicin cause sensitisation of reflex pain behaviours in naïve animals and enhance the sensitisation already present following nerve injury[46], while formalin itself is clearly noxious[86]. TRPA1 can be activated by icilin, although less potently and more slowly than TRPM8, possibly by an indirect route[32].

A number of other channels have also been proposed to be involved in cold transduction and the function of cold-sensitive afferents[45, 68, 88-92], Table 1. Although at present the cold-sensitive TRP channels are the best-studied and perhaps the most promising candidates for involvement in sensory cold detection, it seems likely that we still only appreciate a small part of the overall picture.

Conclusions

In conclusion, the cloning of cool/cold-sensitive TRP family channels has provided a clear molecular basis that might explain cool sensation and cold pain. Different temperature thresholds for the main subject of this review, TRPM8, and for TRPA1, which is suggested (but disputed) to respond to more intense cold stimuli, provide a theoretical basis for cooling-induced analgesia in chronic pain states and cold pain respectively. The differential distribution of TRPM8 in non-nociceptive thermosensory afferents as well as in some nociceptive cells may go towards explaining how low doses of TRPM8 activators can cause active analgesia in chronic pain states, whilst TRPM8 can also contribute to cold pain. Studies with antisense deletion of TRPM8 and with TRPM8 knockout mice confirm that TRPM8 activation can elicit analgesia. The underlying mechanism appears to operate in spinal dorsal horn and rely on inhibitory mGlu Group II/III receptors, yet be independent of opioids. TRPM8 is not widely expressed, other than in sensory afferents, but is present in prostate cells and to a lesser extent in bladder epithelium[93, 94]. The TRPM8 channel is overexpressed in prostate malignancy[94, 95], where TRPM8 activation has been shown to lead to increased apoptosis. The limited distribution of TRPM8 in tissues other than sensory afferents and the fact that even currently available TRPM8 activators are effective analgesics by topical cutaneous application as well as local spinal application support the idea that this may represent a viable therapeutic strategy for chronic pain states. Furthermore, these findings emphasise the need for the discovery of more specific TRPM8 agonists/antagonists so that the potential therapeutic role of this target in chronic pain can be fully evaluated.

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Table 1: Candidate mediators of primary afferent responses to cooling.

Channel	Temperature threshold	Reference
TRPM8	19-25°C in heterologous systems 28-30°C in trigeminal ganglion / DRG neurons	27, 28, 29, 30, 45, 47, 92
TRPA1	~17°C	32, 80
Candidates for cold-inhibited K^+ conductances: TREK-1, 2, TRAAK, unidentified background channel	25-31°C	68, 90, 96
Epithelial Na^+ channel (ENaC)	<25°C	88, 92

Figure 1

Schematic plan – hypothetical roles of TRPM8-containing afferents in pain processing

The diagram illustrates only selected subpopulations of afferents. In chronic pain states, repetitive nociceptive input (1) brings about sensitisation of dorsal horn neurons with accentuated responsiveness to both noxious and previously innocuous stimuli that is perceived at higher centres as pain. This sensitised pain state is subject to powerful analgesia elicited by cooling or low dose icilin/menthol through low threshold, non-nociceptive afferents (3) that act centrally through mGlu Group II/III receptors. Intense cold leading to cold pain will activate nociceptors containing TRPA1 and/or other intense cold detectors (1) and additionally any TRPM8-containing afferents that are activated already by even mild cooling. We hypothesise that the minority subpopulation of TRPM8-containing nociceptors (2) may contain lower numbers of TRPM8 channels than the cool afferents (3) and due to this or other factors may require somewhat more intense TRPM8-mediated inputs to activate them. In this way the TRPM8-containing nociceptors (2) would be activated by cold or perhaps high dose icilin/menthol (as opposed to cool or low dose icilin/menthol) and may contribute actively to cold pain.